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EPA Perspectives on Nanoinformatics: Prioritization Based on Potential for Exposure and Toxicity

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Office of Research and Development (ORD)
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Overview

- Incorporating nanomaterials into ToxCast project™
  - EPA nanomaterial research strategy
  - ToxCast project
  - Comptox toxicity and exposure research on nanomaterials
- Databases and tools developed by CompTox
  - ACToR
  - ToxMiner
  - ExpoCast DB and exposure data curation
  - Virtual Tissue Knowledgebase (VT-KB)

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EPA nanomaterial research strategy

June 2009
http://www.epa.gov/nanoscience/files/nanotech_research_strategy_final.pdf

- Four main research themes:
  - Identifying sources, fate, transport, and exposure
  - **Comptox** Understanding human health and ecological effects to inform risk assessments and test methods
  - Developing risk assessment approaches
  - Preventing and mitigating risks

EPA United States Environmental Protection Agency

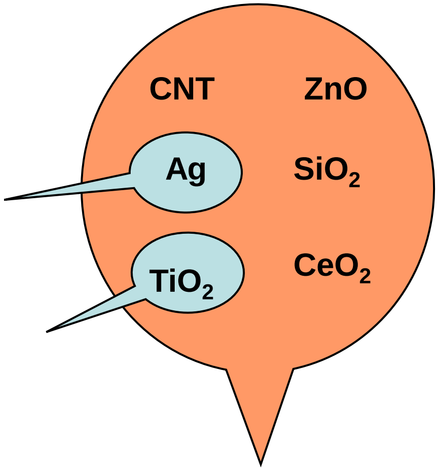
Many nanomaterials to evaluate, but limited time and resources

- Toxicity and exposure research is challenged to keep up with development of novel nanomaterials and applications
- Assesment of nanomaterial (NM) like chemicals is typically case-by-case
- Prioritization of research and screening level assessment of NMs are needed.

Case-by-case examples:

Comprehensive Environmental Assessment approach;
Life cycle assessment;
State of Science Review

Comprehensive Environmental Assessment Approach



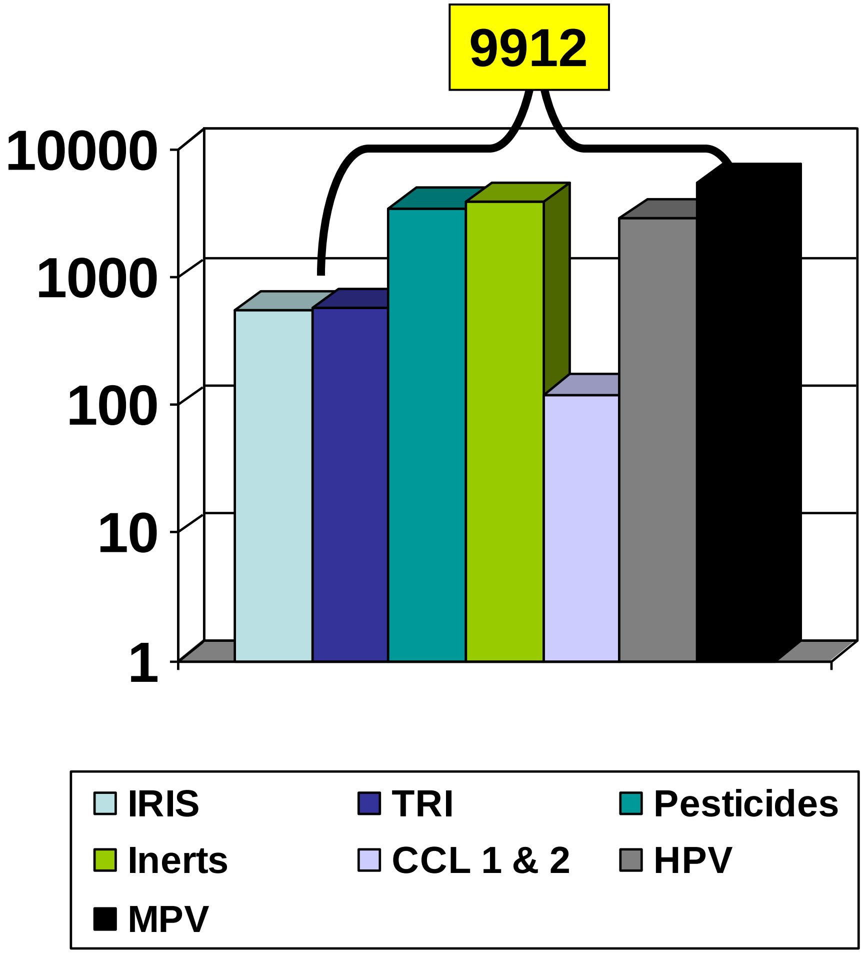
Screening level assessment:

ToxCast™: Bioactivity profiling + exposure potential
(EPA Comptox)

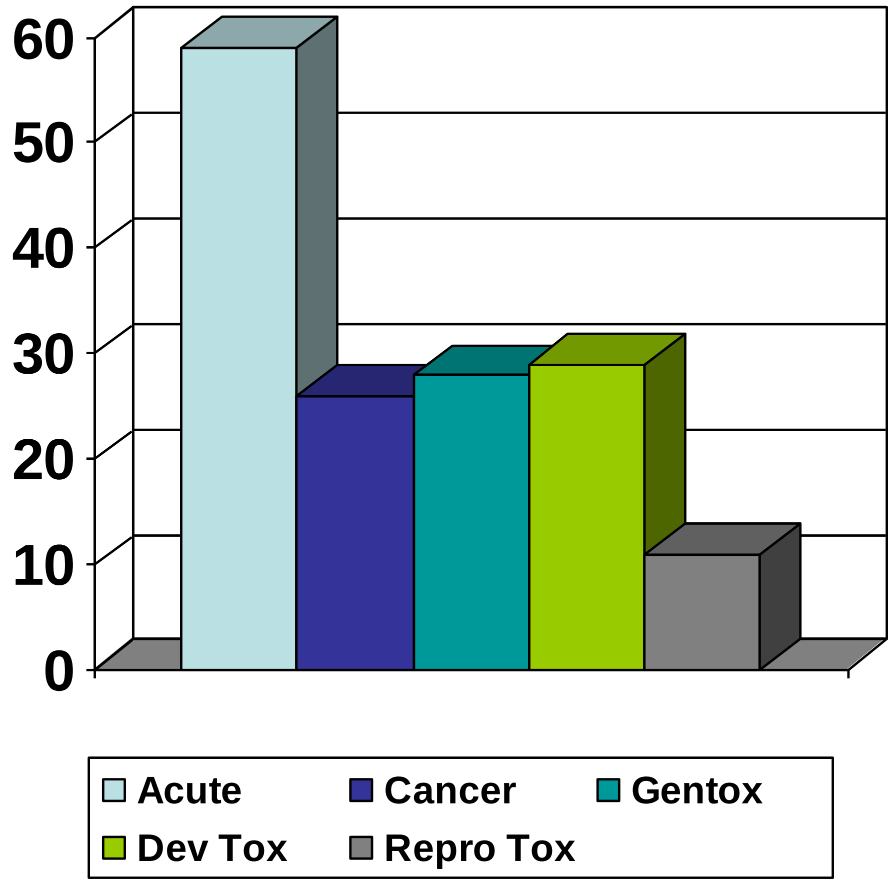
EPA working on larger problem for chemicals

EPA United States Environmental Protection Agency

Too Many Chemicals



Too Little Data (%)

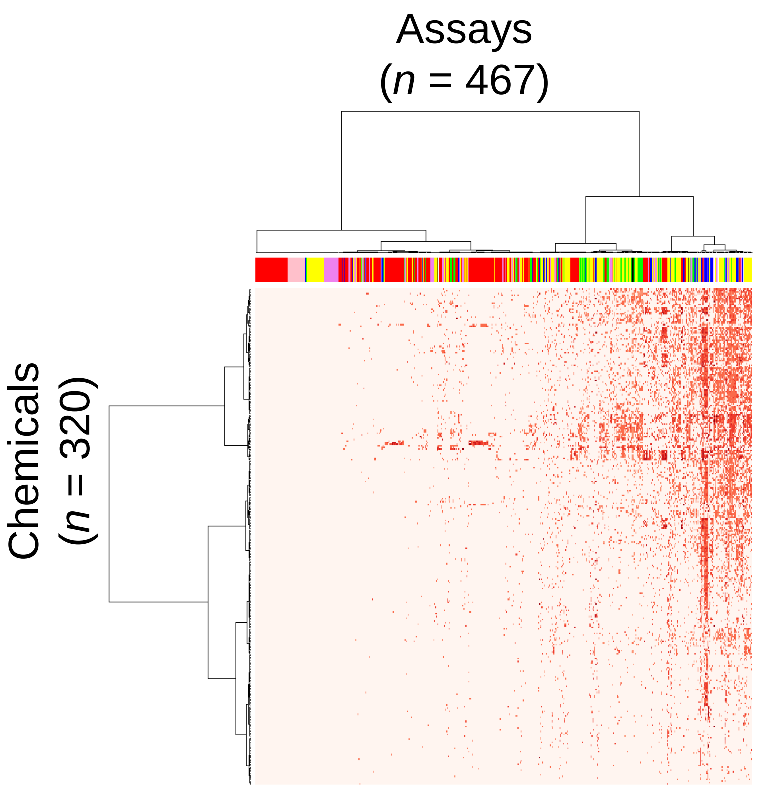


…and costs too much.

Judson et al., 2009, *Environ. Health Perspect*.

ToxCast™ project: Diversity of *in vitro* data from HTS assays

- 500 fast, automated chemical screens (in vitro) generating lots of data
- Phase 1: Screened 300+ well characterized chemicals (primarily pesticides)
- Builds statistical and computer models to forecast potential chemical toxicity



Biochemical Assays

- **Protein families**
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter

Cellular Assays

- **Cell lines**
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- **Primary cells**
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
- **Biotransformation competent cells**
  - Primary rat hepatocytes
  - Primary human hepatocytes
- **Assay formats**
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

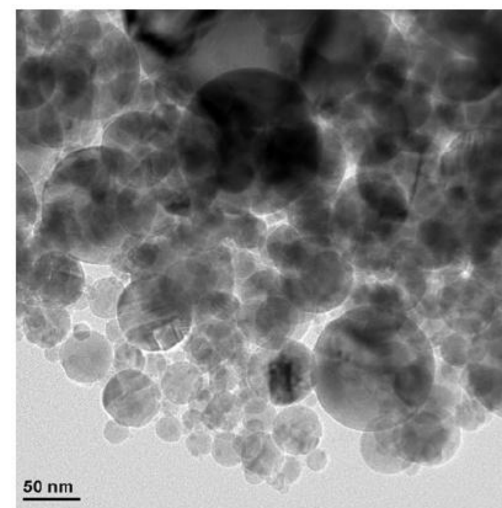
http://www.epa.gov/ncct/toxcast/
Judson et al., 2010, *Environ. Health Perspect*.

Steps to include NMs in ToxCast™

- Classes of NM of interest: Au, Ag, CNT, TiO_2, CeO_2, ZnO, SiO_2

  * Initial pilot materials

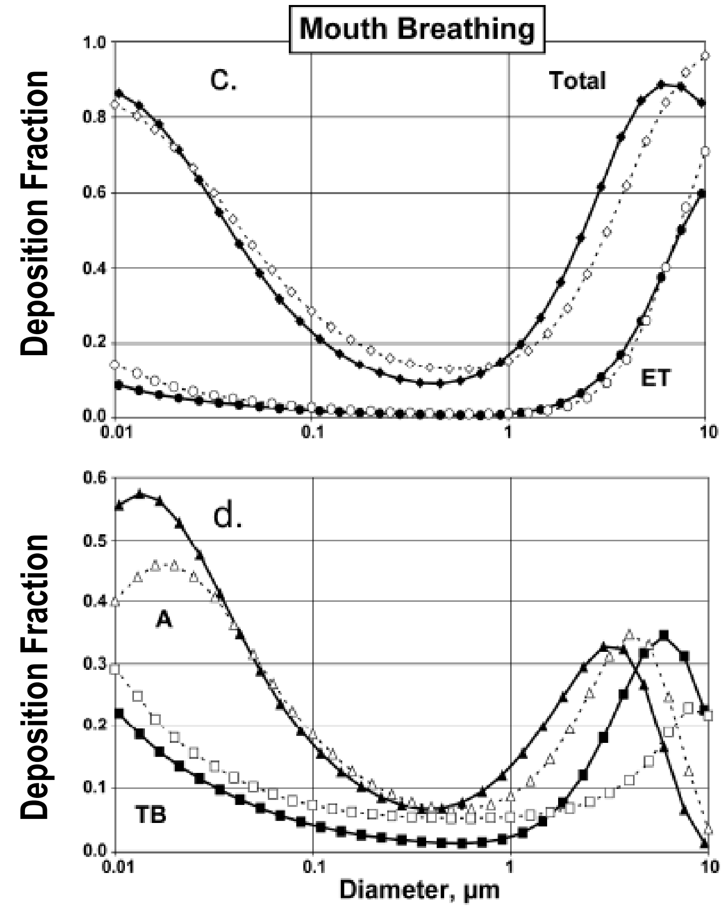
- Major steps:
  - Develop handling protocols
    - Compare protocols used in Center for Environmental Implications of NanoTechnology (CEINT) at Duke Univ., ENPRA, and Japan NIST
  - Determine concentration ranges to test
    - Select based on potential for real world human exposures
  - Characterize NMs
    - CEINT at Duke Univ.
  - Perform High-throughput screening (HTS)
    - Analyze HTS data and apply ToxCast methodology



TEM image of TiO_2

Using Multiple-path particle dosimetry model to determine concentrations

- Open-source computational MPPD modeling tool (Applied Research Associates)
  - Calculates human respiratory tract particle deposition/clearance after inputing NM aerosol conc.
- Reviewed literature on NM aerosol concentrations in occupational settings
  - Typically $\leq$ 0.1 mg/m^3 for TiO_2, Ag, CNTs
- Performed sensitivity analysis
  - Most important inputs: aerosol concentration, breathing conditions (heavy, light exercise, rest), aspect ratio (for CNTs)

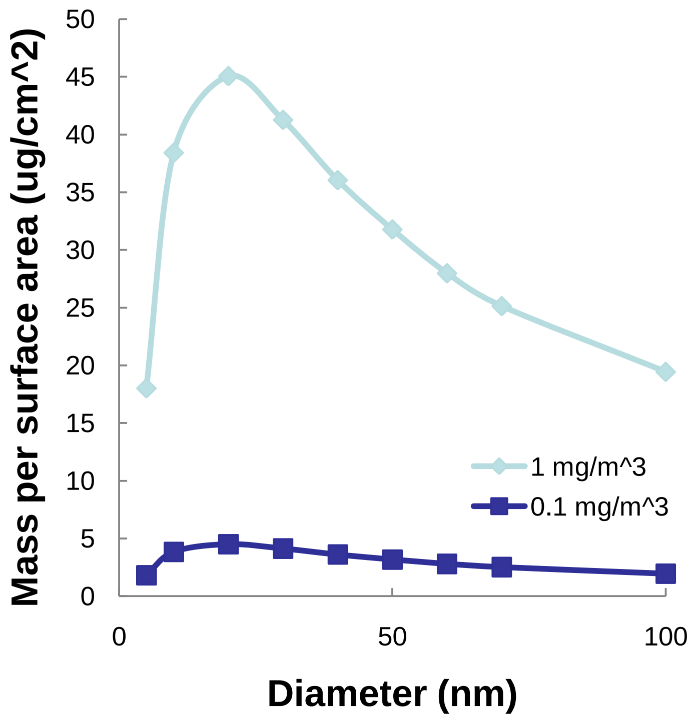


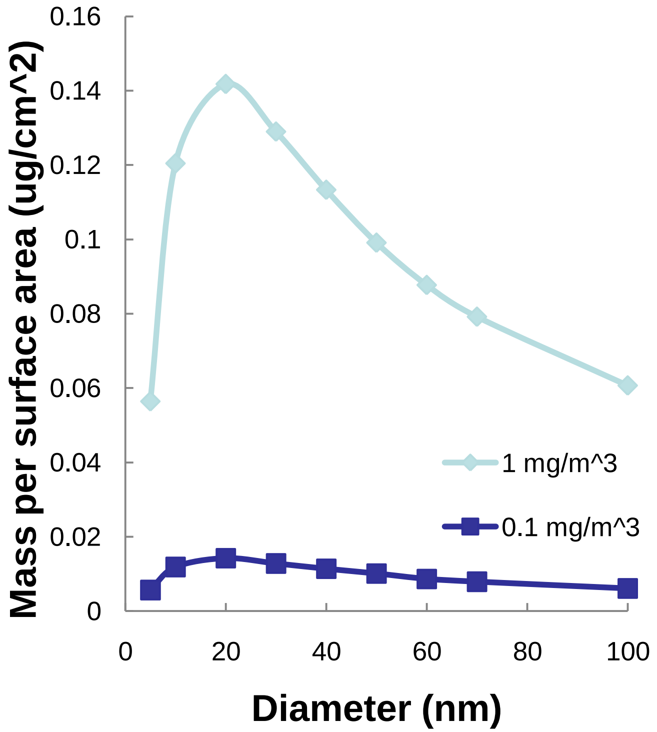
ICRP
MPPD
Light exercise breathing conditions

Integrated Science Assessment for Particulate Matter, Dec. 2009, EPA NCEA, Jim Brown

NM alveolar mass retained in human lungs

- Ag & TiO_2 nanoparticles





- Exposure duration: Full working lifetime of 45 years (8 h/day, 5 days/week)
- Exposure duration: 24 hours

Alveolar mass retained for a full working lifetime to 1 mg/m^3 → Similar to high-end doses (~ 100-200 ug/mL) typical of *in vitro* testing

NM physicochemical characterization

- Collaboration with Center for Environmental Implications of NanoTechnology (CEINT) at Duke Univ.

All NMs

- As received (dry powder or suspension)
- In stock (prepared per OECD protocol: sonication in water with 2% serum)

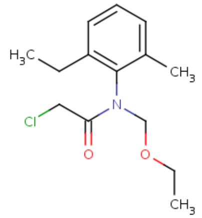
Selected NM medium/cell combination

- In testing mediums
- *In situ* (cell/tissue)

- Size distribution, shape
  (TEM DLS Cytovita)
- Surface area
  (BET Calculation from DLS)
- Chemical composition,crystal form
  (XRD Possibly ICP-MS)
- Rate of dissolution
  (ICP-AES Possibly ion specific probe)
- Surface composition/contamination
  (TOC Possibly SEM+EDS)
- Surface charge, zeta potential
  (Zetasizer)
- Possibly hydrophobicity, surface redox react.

Developing screening models

Animal Study → Toxicity



In vitro assay → Pathway Perturbation

Connections are made by looking for statistical associations across many chemicals

Requires both *in vitro* and *in vivo* data

Once a model is "qualified":

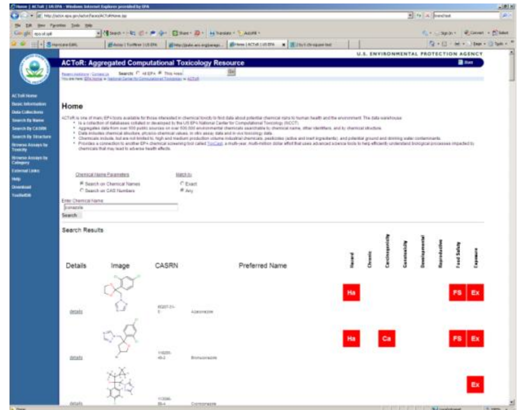
- New chemicals (nanomaterials) can be run through assays
- Results of assays can be used to rank chemicals (nanomaterials) for potential to cause toxicity

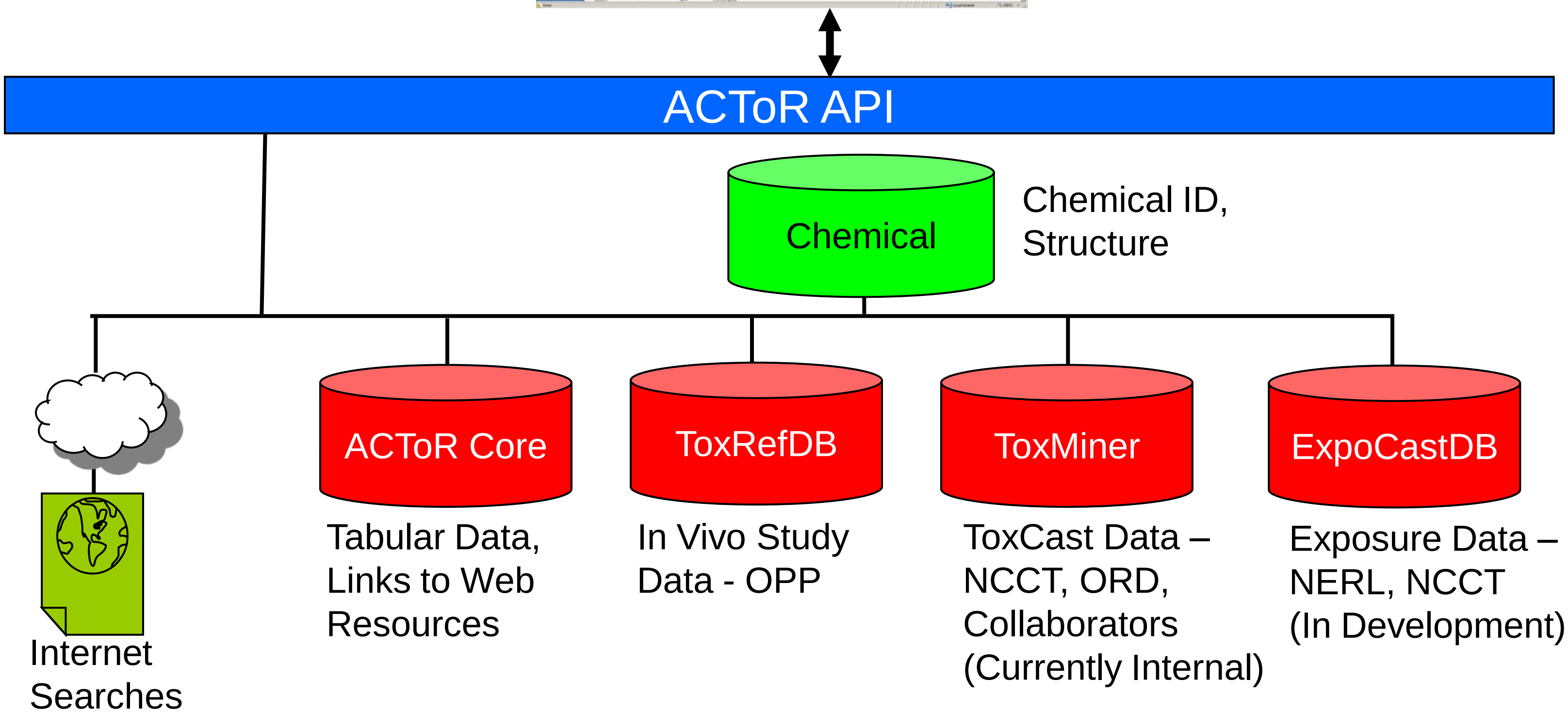
NCCT databases and tools

- Aggregated Computational Toxicology Resource (ACToR)
  - Database to find chemical toxicity info from large number of sources.
- ToxMiner
  - Database to house detailed data ToxCast and ToxRefDB - used for ToxCast analyses.
- ExpoCast DB
  - Detailed chemical concentration by media data from observational exposure studies.
- Virtual tissue Knowledge base (VT-KB)
  - Tool developed to curate literature on chemical toxicity

ACToR: Aggregated Computational Toxicology Resource

http://actor.epa.gov/





ACToR goals and data sources

- Compile all publicly available information on environmental chemicals

Category	Count
Data Sets	580
Chemicals	546,956
Assays	3,213
Assay Components	7,221
Data Points	6,662,296

- EPA (OPP, OPPT, NCEA, NERL)
- FDA, NIH, CDC, OSHA, USDA
- States and other countries
- Universities
- NGOs
- Companies

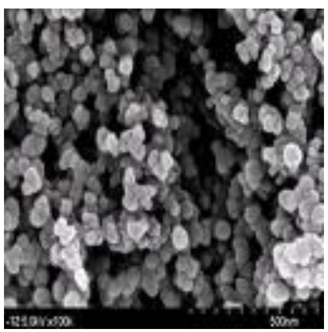
- Make data available for downloading, data mining
  - Available through data.gov
  - Entire DB can be downloaded and installed locally
- Make it easy to see data gaps
  - Provides resource for EPA testing programs
- Make it widely used
  - over 2000 regular users

Store NM physicochemical properties

Nanomaterial identity

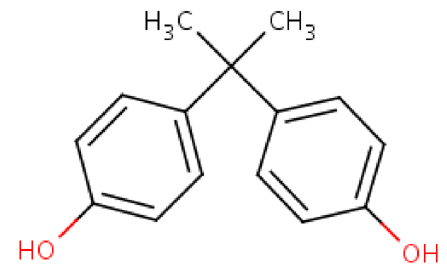
Nanomaterials require the same types of naming conventions

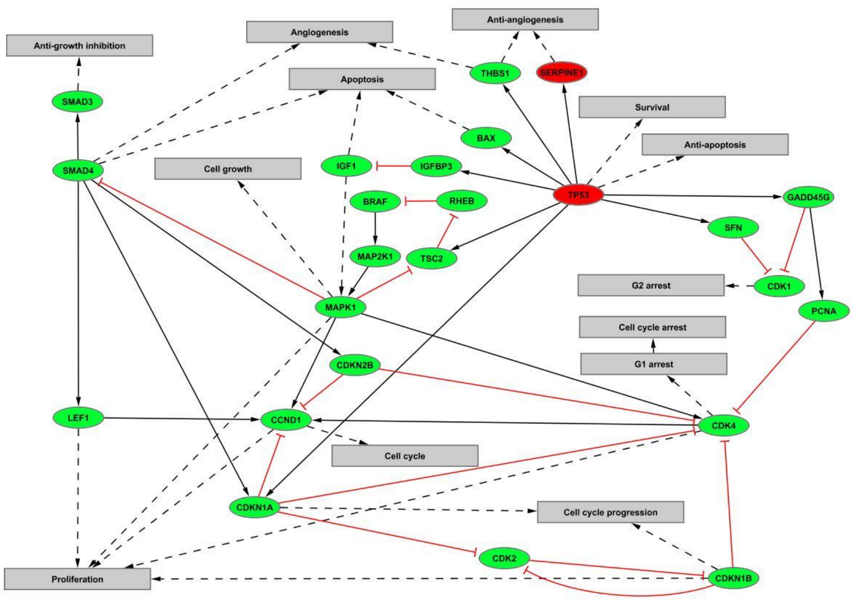
- Common names
- Systematic names
- Computable representation of structure
- Open source CASRN-like "code" for linking data from many sources



ToxMiner – ToxCast Data

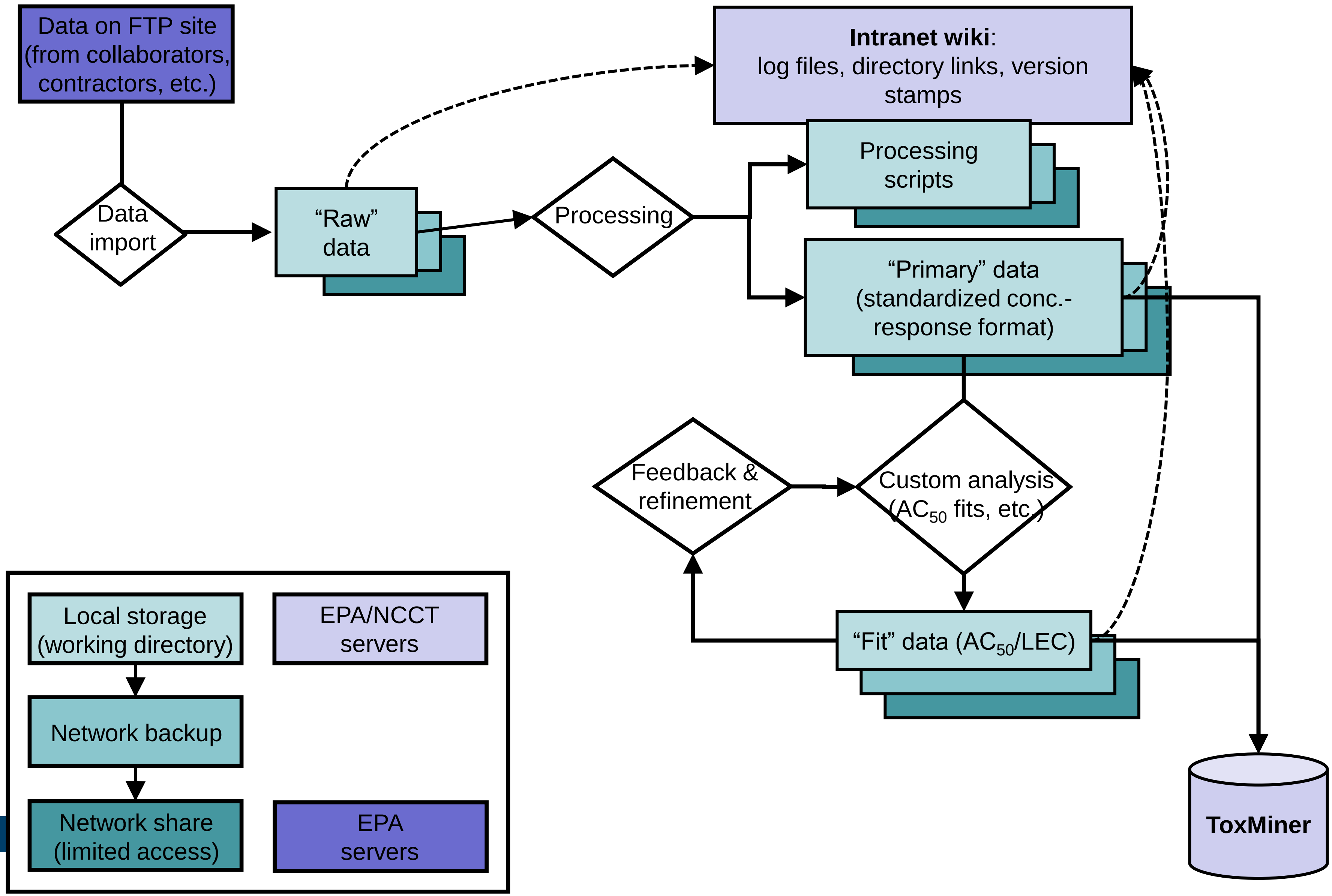
- Links
  - Chemicals
  - Assays
  - Genes
  - Pathways
  - Endpoints
- Allows data analyses
  - Statistical associations (R-script)
  - Biologically driven data mining





Store in vitro HTS assay data on NMs

ToxCast assay data workflow



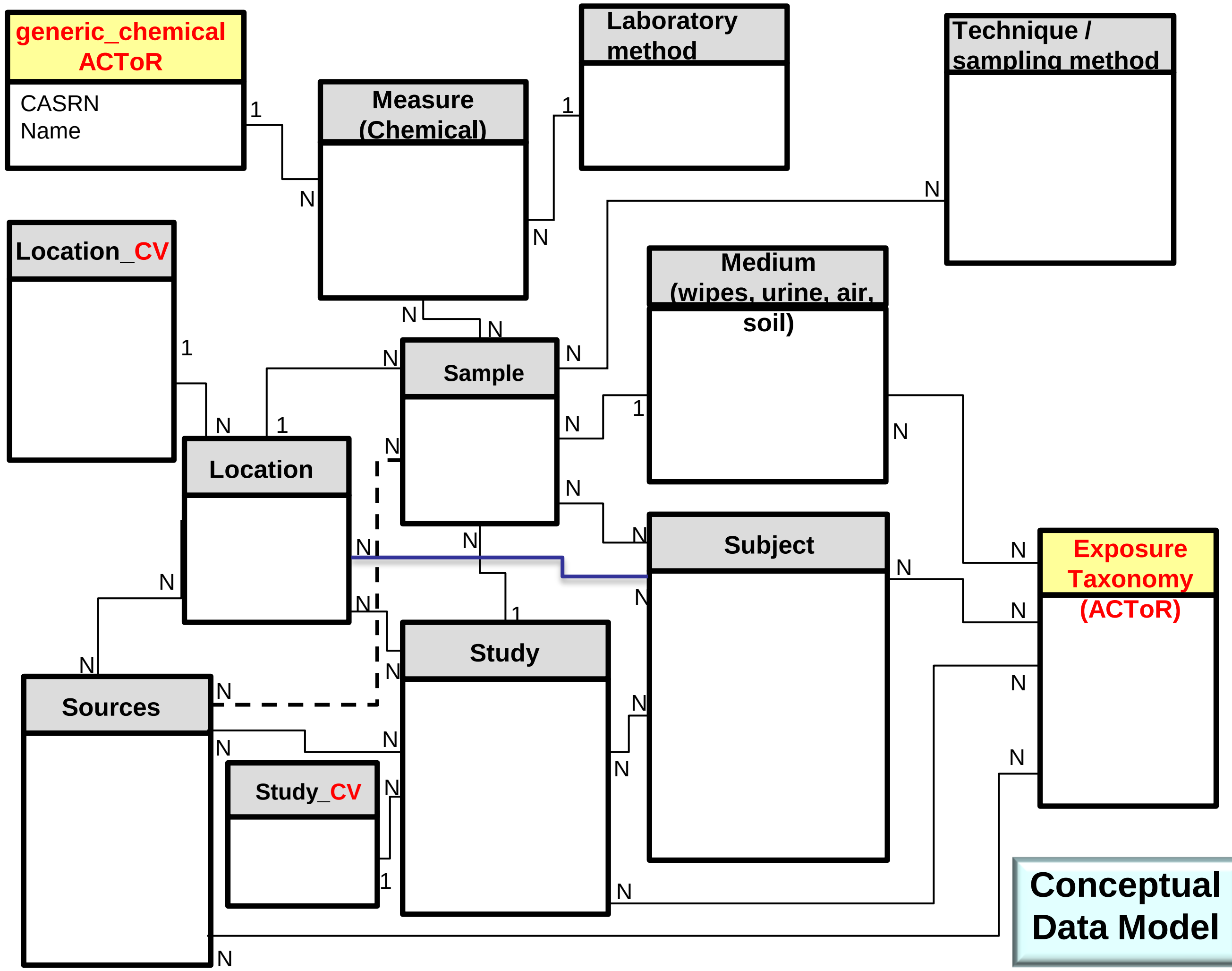
ExpoCast DB

- Data from NERL studies
1) American Health Home Survey
2) HUD Child Care Center Survey (“CCC”)
3) CTEPP – NC
4) CTEPP – OH

- Full raw data sets available to download

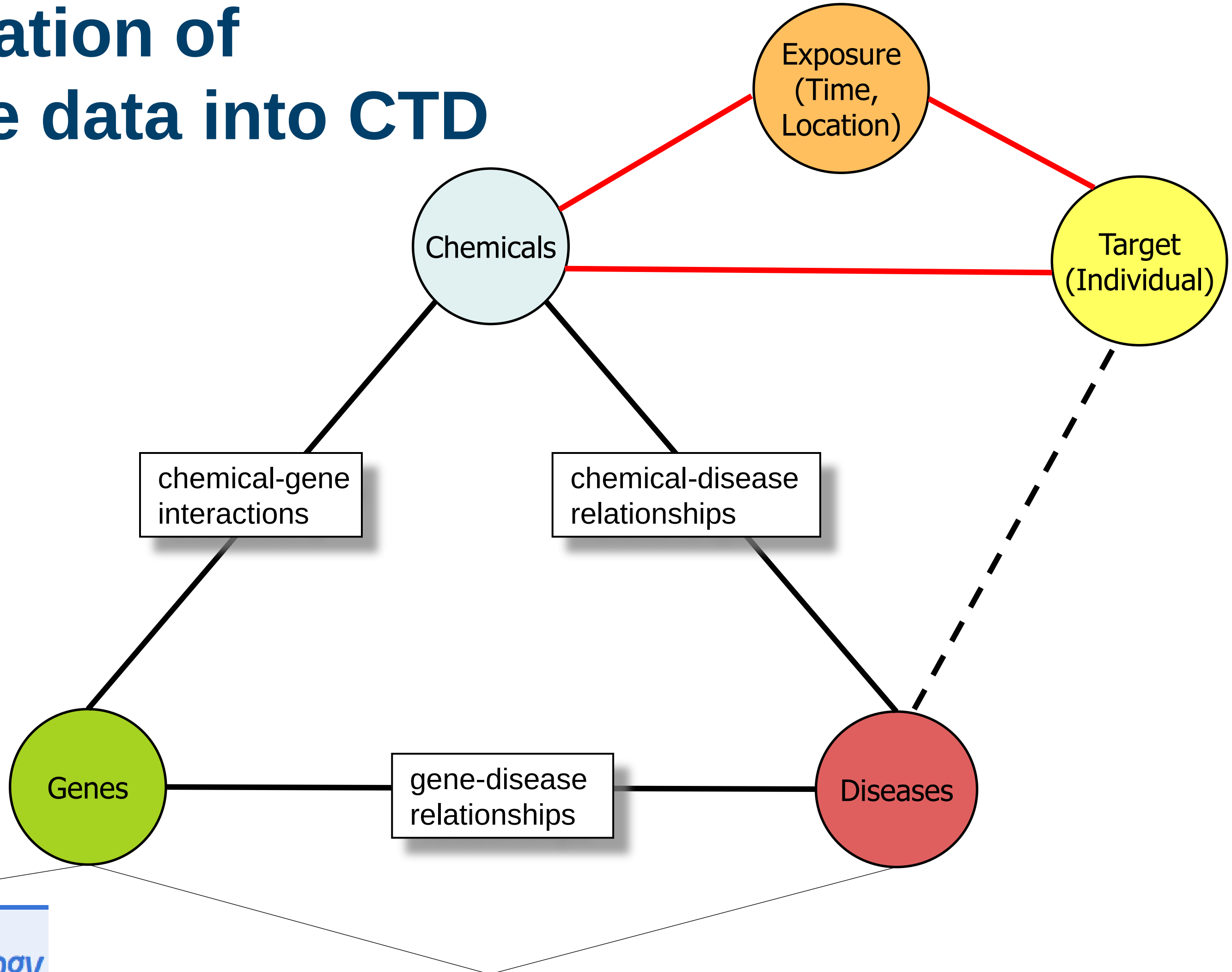
- Browse data capability
- By study name, chemical list, media list

- Descriptive statistics capabilities



Store exposure aerosol concentrations of NM in occupational settings

Pilot curation of exposure data into CTD



the Gene Ontology

functional annotations

pathway data

CTD advances understanding of the effects of environmental chemicals on human health.

The Comparative Toxicogenomics Database™

Summary

- Results of nanomaterial ToxCast screening and physicochemical characterization will be publicly accessible through ACToR
- For chemicals, informatics infrastructure is in place for:
  - Capturing chemical identity
  - Capturing *in vitro* and *in vivo* data
  - Measuring and modeling biotransformation / metabolism
  - Building statistical and biologically-based models
  - Prioritizing chemicals for targeted testing
  - Dealing with 10^4 to 10^6 chemicals

- Challenges for nanomaterials
  - Material identity
  - Quantification of imaging characterization results

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